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**From:** Qazi, Sabiha  
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Please provide following references.

Wargovich, Michael J. et al., Carcinogenesis, (2000), 21(6), 1149-1155. Please get the date of publication.

White, E. Lucile et al., Oncol. Rep. (1998), 5(3), 717-722.

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Sabiha Qazi

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## Efficacy of potential chemopreventive agents on rat colon aberrant crypt formation and progression

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We assessed the effects of 78 potential chemopreventive agents in the F344 rat using two assays in which the inhibition of carcinogen-induced aberrant crypt foci (ACF) in the colon was the measure of efficacy. In both assays ACF were induced by the carcinogen azoxymethane (AOM) in F344 rats by two sequential weekly injections at a dose of 15 mg/kg. Two weeks after the last AOM injection, animals were evaluated for the number of aberrant crypts detected in methylene blue stained whole mounts of rat colon. In the initiation phase protocol agents were given during the period of AOM administration, whereas in the post-initiation assay the chemopreventive agent was introduced during the last 4 weeks of an 8 week assay, a time when ACF had progressed to multiple crypt clusters. The agents were derived from a priority listing based on reports of chemopreventive activity in the literature and/or efficacy data from *in vitro* models of carcinogenesis. During the initiation phase carboxyl amidoimidazole, *p*-chlorophenylacetate, chlorpheniramine maleate, D609, diclofenac, etoperidone, eicosatetraynoic acid, farnesol, ferulic acid, lycopene, meclizine, methionine, phenylhexylisothiocyanate, phenylbutyrate, piroxicam, 9-cis-retinoic acid, S-allylcysteine, taurine, tetracycline and verapamil were strong inhibitors of ACF. During the post-initiation phase aspirin, calcium glucarate, ketoprofen, piroxicam, 9-cis-retinoic acid, retinol and rutin inhibited the outgrowth of ACF into multiple crypt clusters. Based on these data, certain phytochemicals, antihistamines, non-steroidal anti-inflammatory drugs and retinoids show unique preclinical promise for chemoprevention of colon cancer, with the latter two drug classes particularly effective in the post-initiation phase of carcinogenesis.

### Introduction

We have previously reported on the efficacy of a number of natural and synthetic compounds as potential chemopreventives for colon cancer using the induction of aberrant crypt foci (ACF) as the primary end-point (1). ACF are preneoplastic lesions in rat colon (2–4). They are induced by all known

**Abbreviations:** ACF, aberrant crypt foci; AOM, azoxymethane; CAI, carboxyl amidoimidazole; ETYA, eicosatetraynoic acid; NSAID, non-steroidal anti-inflammatory drug; PHITC, phenylhexylisothiocyanate.

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colon carcinogens and exhibit a number of molecular mutations in regulatory genes consonant with the development of human colon cancer, most notably in the *ras* oncogene and *apc* tumor suppressor gene (5,6). In our initial study we only evaluated the potential chemopreventive agents during the initiation phase of carcinogenesis. We recognized that compounds acting in the post-initiation period could result in false negatives in the assay. With this in mind we developed a post-initiation protocol for the evaluation of potential chemopreventive agents on established ACF in the rat colon and found that certain non-steroidal anti-inflammatory drugs (NSAIDs) were powerful suppressors of aberrant crypt growth and progression (7). This protocol clearly has more clinical relevance since it may identify candidate agents that prove useful in preventing the recurrence and progression of precursor lesions for colon cancer. In the current study we report on the further evaluation of 78 agents for the prevention of colon cancer. Specifically, we have evaluated the activity of 30 agents in the post-initiation protocol. The data suggest that two classes of drugs, the NSAIDs and retinoids, act strongly in the post-initiation phase of carcinogenesis of the colon.

### Materials and methods

#### Animals, diets, test agents and carcinogens

Male F344 rats were purchased at 6 weeks of age from Harlan Sprague-Dawley (Wilmington, MA), were quarantined for 5 days and then housed in standard cages and with standard bedding in the animal facility on a 12 h light-dark cycle, at 50% relative humidity, with continual access to drinking water. At 7 weeks of age, all rats were fed the AIN-76A diet (Dyets Inc., Bethlehem, PA) on which they remained for the duration of the experiment. For each agent to be tested, 40 rats were randomized into groups of 10. The groups were: (i) negative control (no test agent, no carcinogen); (ii) positive control (no test agent, with carcinogen); (iii) and (iv) treatment groups (test agent and carcinogen). The positive and negative controls were fed the standard AIN-76A diet throughout the experiment duration. The treatment groups had either 40 or 80% of the maximum tolerated dose of each agent included in their diets; these doses are known not to cause a decrement in body weight over a 5 week experimental period. Doses were chosen based on communication with the National Cancer Institute, published data or proprietary information. In the initiation protocol (protocol A) (Figure 1), the diets were fed to the rats beginning 1 week prior to injection with the carcinogen or saline, then continuously for the next 4 weeks. In the post-

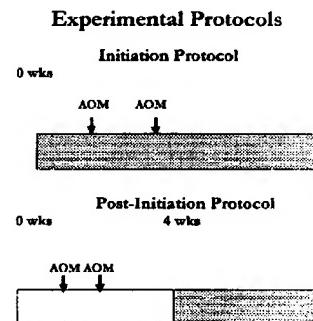


Fig. 1. Experimental schema. Shading indicates phase when test agent was present in the experimental diet.

**Table I. Effect of test agents on AOM-induced ACF in rat colon during the initiation phase**

Agent (protocol)	Dose (g/kg diet)	Aberrant crypts/colon		
		Mean ± SEM	Percent of control	Result
Amiloride (A)	0	160 ± 5	100	
	0.05	134 ± 16	84	-
	0.1	205 ± 40	128	-
Black tea extract (A)	0	73 ± 3	100	
	4.5	87 ± 14	119	-
	9	100 ± 10	137	+*
Black tea polyphenols (A)	0	132 ± 4	100	
	0.36 g/l <sup>b</sup>	98 ± 10	74	+*
	1.2 g/l <sup>b</sup>	114 ± 10	86	-
Bromoergocryptine (A)	0	98 ± 3	100	
	400 µg	105 ± 5	107	-
	800 µg	92 ± 6	94	-
2-n-Butylthiophene (A)	0	240 ± 7	100	
	0.05	208 ± 18	87	-
	0.1	295 ± 18	123	+*
CAI (A)	0	116 ± 3	100	
	0.1	59 ± 5	51	+*
	0.2	49 ± 14	42	+*
Carbenoxolone (A)	0	240 ± 7	100	
	0.6	299 ± 12	125	+*
	1.2	304 ± 22	127	+*
p-Chlorophenylacetate (A)	0	132 ± 4	100	
	1	95 ± 7	72	+*
	2	91 ± 9	69	+*
Chlorpheniramine maleate (A)	0	103 ± 3	100	
	0.4	73 ± 2	71	+*
	0.8	73 ± 5	71	+*
Cimetidine (A)	0	160 ± 5	100	
	0.25	158 ± 8	99	-
	0.5	148 ± 8	93	-
Conjugated linoleic acid (A)	0	79 ± 3	100	
	5	97 ± 9	123	-
	10	92 ± 7	116	-
D609 (A)	0	132 ± 4	100	
	0.15	58 ± 4	44	+*
	0.30	80 ± 8	61	+*
Diclofenac (A)	0	120 ± 5	100	
	0.06	84 ± 7	70	+*
	0.13	85 ± 7	71	+*
Epigallocatechin gallate (A)	0	132 ± 4	100	
	0.36 g/l <sup>b</sup>	110 ± 12	83	-
	1.2 g/l <sup>b</sup>	86 ± 8	65	+*
Esculetin (A)	0	240 ± 7	100	
	2.5	203 ± 10	85	-
	5	242 ± 19	101	-
ETYA (A)	0	103 ± 4	100	
	0.08	75 ± 2	73	+*
	0.16	75 ± 3	73	+*
Etoperdione (A)	0	90 ± 3	100	
	1.25 mg	70 ± 5	78	+*
	2.5 mg	49 ± 4	54	+*
Farnesol (A)	0	90 ± 3	100	
	0.8	64 ± 7	71	+*
	1.6	63 ± 5	70	+*
Ferulic acid (A)	0	103 ± 3	100	
	10	61 ± 3	59	+*
	20	80 ± 5	78	+*
Geranyl geraniol (A)	0	160 ± 5	100	
	0.8	120 ± 10	75	+*
	1.6	175 ± 10	109	-
Glycine (A)	0	240 ± 7	100	
	10	223 ± 11	93	-
	20	278 ± 24	116	-

**Table I. Cont.**

Agent (protocol)	Dose (g/kg diet)	Aberrant crypts/colon		
		Mean ± SEM	Percent of control	Result
Green tea extract (A)	0	120 ± 5	100	
	4.5	137 ± 8	114	-
	9	144 ± 10	120	a
Green tea polyphenols (A)	0	120 ± 5	100	
	0.36 g/l <sup>b</sup>	129 ± 9	108	-
	1.2 g/l <sup>b</sup>	101 ± 7	84	-
Lycopene (A)	0	132 ± 4	100	
	0.038	87 ± 8	66	+c
	0.075	61 ± 3	46	+c
Meclizine (A)	0	91 ± 3	100	
	0.3	53 ± 5	58	+c
	0.6	50 ± 5	55	+c
Meclofenamate sodium (A)	0	103 ± 4	100	
	0.02	129 ± 11	125	a
	0.04	137 ± 11	133	a
Melatonin (A)	0	73 ± 3	100	
	1 mg	62 ± 9	85	-
	2 mg	77 ± 8	105	-
l-Methionine (A)	0	240 ± 7	100	
	8	186 ± 10	78	+c
	16	198 ± 13	83	+c
p-Methoxyphenol (A)	0	103 ± 4	100	
	0.4	136 ± 9	132	a
	0.8	105 ± 1	102	-
Nerolidol (A)	0	79 ± 3	100	
	2.5	138 ± 10	175	a
	5	60 ± 6	76	+c
Nicotinic acid (A)	0	103 ± 4	100	
	0.08	103 ± 8	100	-
	0.4	106 ± 6	103	-
1,2-Oxothiazolidine 4-carboxylate (A)	0	79 ± 3	100	
	2	136 ± 10	172	a
	4	130 ± 10	165	a
Perillyl alcohol (A)	0	160 ± 5	100	
	1.25	146 ± 8	91	-
	2.5	157 ± 10	98	-
PHITC (A)	0	91 ± 3	100	
	1 mg	65 ± 5	71	+c
	0.5 mg	57 ± 4	63	+c
Phenylbutyrate (sodium) (A)	0	132 ± 4	100	
	1	73 ± 6	55	+c
	2	103 ± 11	78	+c
Phloretin (A)	0	240 ± 7	100	
	2	207 ± 15	86	-
	4	267 ± 16	111	-
Piroxicam (A)	0	98 ± 3	100	
	0.2	48 ± 3	49	+c
	0.4	32 ± 2	33	+c
Polyvinylpyrrolidone (A)	0	98 ± 3	100	
	10	86 ± 4	88	-
	20	90 ± 4	92	-
Promethazine (A)	0	160 ± 5	100	
	0.025	170 ± 12	106	-
	0.05	155 ± 10	97	-
Quinacrine HCl (A)	0	103 ± 4	100	
	0.1	150 ± 12	146	a
	0.3	106 ± 7	103	-
9-cis-retinoic acid (A)	0	73 ± 3	100	
	0.19	45 ± 6	62	-c
	0.39	36 ± 6	49	+c
Ro25-6760 (A)	0	104 ± 7	100	
	0.63 µg >	86 ± 9	83	-
	1.27 µg >	118 ± 11	114	-

Table I. Cont.

Agent (protocol)	Dose (g/kg diet)	Aberrant crypts/colon		
		Mean $\pm$ SEM	Percent of control	Result
S-allylcysteine (A)	0	84 $\pm$ 4	100	
	0.125	57 $\pm$ 7	68	+ <sup>c</sup>
	0.25	39 $\pm$ 3	46	+ <sup>c</sup>
SC58635 (A)	0	79 $\pm$ 3	100	
	0.56	76 $\pm$ 5	96	-
	1.13	76 $\pm$ 9	96	-
d,l-Selenomethionine (A)	0	73 $\pm$ 3	100	
	1 mg	78 $\pm$ 9	107	-
	2 mg	72 $\pm$ 6	99	-
Stevioside (A)	0	91 $\pm$ 3	100	
	0.3	70 $\pm$ 5	77	+ <sup>c</sup>
	0.6	63 $\pm$ 5	69	+ <sup>c</sup>
Taurine (A)	0	85 $\pm$ 4	100	
	0.6	63 $\pm$ 6	74	+ <sup>c</sup>
	1.2	31 $\pm$ 3	36	+ <sup>c</sup>
Tetracycline (A)	0	103 $\pm$ 3	100	
	1.2	57 $\pm$ 5	55	+ <sup>c</sup>
	12.5	37 $\pm$ 3	36	+ <sup>c</sup>
Theophylline (A)	0	91 $\pm$ 3	100	
	2	72 $\pm$ 8	79	-
	4	88 $\pm$ 9	97	-
Tolmetin (A)	0	79 $\pm$ 3	100	
	0.38	50 $\pm$ 6	63	+ <sup>c</sup>
	0.75	61 $\pm$ 7	77	+ <sup>c</sup>
Triprolidine (A)	0	91 $\pm$ 3	100	
	0.75	46 $\pm$ 5	51	+ <sup>c</sup>
	1.5	54 $\pm$ 3	59	+ <sup>c</sup>
Ursolic acid (A)	0	73 $\pm$ 3	100	
	38 mg	85 $\pm$ 10	116	-
	75 mg	79 $\pm$ 6	108	-
Verapamil (A)	0	103 $\pm$ 3	100	
	0.6	85 $\pm$ 4	83	+ <sup>c</sup>
	1.2	57 $\pm$ 3	55	+ <sup>c</sup>
WR151327 (A)	0	137 $\pm$ 5	100	
	2.5	87 $\pm$ 6	64	+ <sup>c</sup>
	5	57 $\pm$ 5	42	+ <sup>c</sup>

<sup>a</sup>Significantly higher than control group,  $P < 0.05$ .<sup>b</sup>Tea compounds given in drinking water.<sup>c</sup>+, significant inhibition compared with control group,  $P < 0.05$ .

initiation protocol (protocol B) (Figure 1), the test agent was administered beginning at week 5 and administered continuously in the feed until the end of week 8, when the animals were killed. The test agents used in the study were procured at the highest available purity from a variety of sources: amiloride, aspirin, bromoergocryptine, calcium glucarate, *p*-carbenoxalone, chlorpheniramine maleate, 13-*cis*-retinoic acid, cimetidine, farnesol, ferulic acid, folic acid, geranyl geranioyl, glycine, ibuprofen, meclizine, meclofenamate, melatonin, *l*-methionine, *p*-methoxyphenol, nicotinic acid, piroxicam, polyvinylpyrrolidone, promethazine, quinacrine HCl, retinol, rutin, *d,l*-selenomethionine, stevioside, taurine, tetracycline, theophylline, tolmetin, all-trans-retinoic acid, triprolidine, ursolic acid and verapamil were purchased from Sigma Chemical Co. (St Louis, MO). 2-*n*-Butylthiophene was purchased from Lancaster Synthesis Ltd (Windham, NH). Esculetin, nerolidol and 1,2-oxothiazolidine 4-carboxylate were purchased from Aldrich Chemical Co. (Milwaukee, WI). Eicosatetraenoic acid (ETYA) was purchased from Biomol Inc. (Plymouth Meeting, PA). Diallyl disulfide was purchased from Pfaltz and Bauer Inc. (Waterbury, CT). Perillyl alcohol was purchased from CTC Organics (Atlanta, GA). Several companies provided their reagents as gifts for research: S-allylcysteine (Wakunaga Pharmaceutical Co., Hiroshima, Japan), difluoromethylornithine (Marion-Merrill Dow, Cincinnati, OH), ketoprofen (Rhone-Poulenc, Vitry-Sur-Seine, France), Ro16-9100 (Hoffman-LaRoche Inc., Nutley, NJ), sulindac and its metabolites (Cell Pathways, Aurora, CO) and tea extracts (Lipton, Englewood Cliffs, NJ). All other agents were provided by the National Cancer Institute Chemical Repository. For several agents random spot checks on the stability of the test agent in the diet were performed using HPLC, but

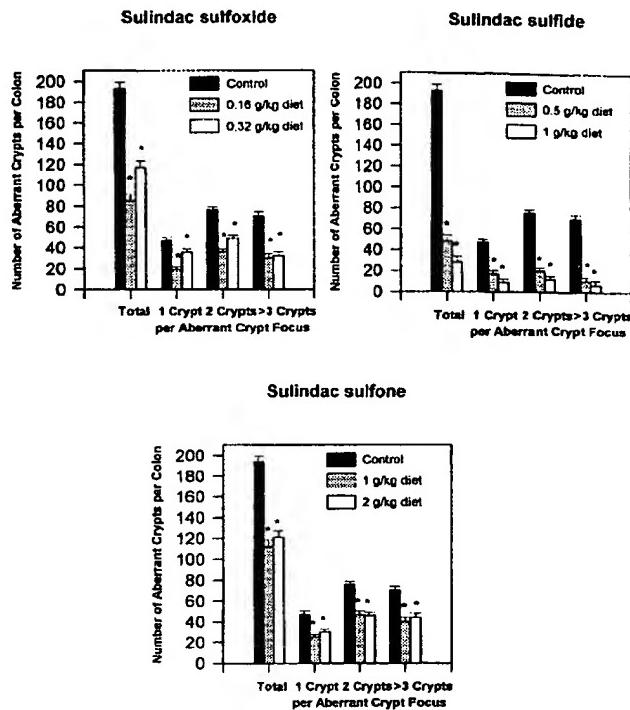


Fig. 2. Effect of sulindac and sulindac metabolites on ACF progression. Compounds were given in the diet post-initiation following AOM. Values are means  $\pm$  SD. \*Significantly different from the control at  $P < 0.05$ .

not for all agents tested. All diets were made fresh weekly and held at  $-20^{\circ}\text{C}$  prior to placement in the animal diet jars.

For both protocols, the positive control group and the two treatment groups were injected i.p. with the carcinogen azoxymethane (AOM) purchased from Ash Stevens Inc. (Detroit, MI) twice weekly (weeks 2 and 3 of each experiment) at a dose level of 15 mg/kg body wt. The negative control group was injected with saline in place of AOM. At the end of week 5 (initiation protocol A) and at the end of week 8 (post-initiation protocol B) of each experiment the rats were killed by  $\text{CO}_2$  asphyxiation and the colon removed for evaluation of aberrant crypts. This study was approved by the Institutional Animal Care and Use Committee at The University of Texas M.D. Anderson Cancer Center (protocol 11-8807832).

#### Aberrant crypt assay

The colons were removed and flushed with cold phosphate-buffered saline, then cut open along the longitudinal median and fixed flat in 10% buffered formalin for 24 h. The method of Tudek *et al.* (4) was used to stain and highlight ACF. For each test agent the number of ACF was evaluated in the 0.3% methylene blue stained colon by two scorers unaware of the treatment assignment. We scored ACF under 40 $\times$  magnification using a Nikon dissecting microscope with a fiber optic light source to transilluminate the colon. The study consisted of multiple experiments. Experiments usually involved the testing of four compounds at a particular time over a period of 18 months. The number of ACF in the AOM-treated controls ranged from 73 to 240 over the course of the study, but efficacy was always assessed against the AOM controls within each experimental set.

#### Statistical analysis

All data were analyzed using SigmaStat software (SPSS, Chicago, IL). Both treatment doses were compared with the AOM-only group using one-way ANOVA. If a significant difference ( $P < 0.05$ ) was observed we used the Bonferroni *t*-test as a multiple comparison test. The data were also tested for normality; if the data were not normally distributed we used the non-parametric Kruskal-Wallis test for multiple comparisons.

#### Results

Table I lists the agents that we evaluated in the initiation protocol, which tested for chemopreventive effects during the

**Table II.** Effect of test agents on AOM-induced aberrant crypt foci in rat colon during the post-initiation phase

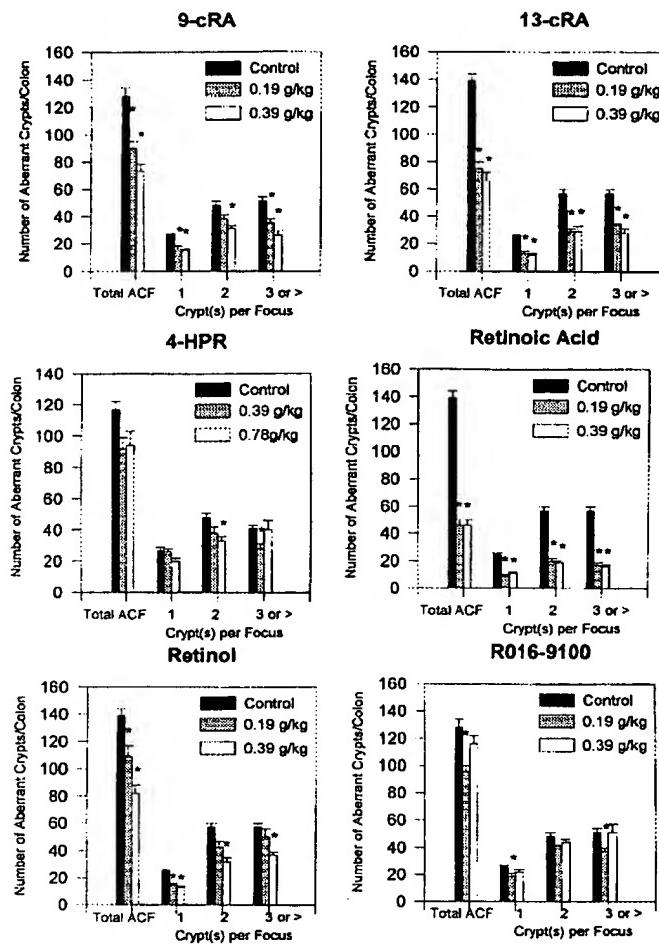
Agent (protocol)	Dose (g/kg diet)	Aberrant crypts/colon		
		Mean ± SEM	Percent of control	Result
Anethole trithione (B)	0	182 ± 8	100	-
	0.1	177 ± 16	97	+ <sup>b</sup>
	0.2	106 ± 13	58	+ <sup>b</sup>
Aspirin (B)	0	175 ± 7	100	-
	0.2	136 ± 5	78	+ <sup>b</sup>
	0.4	103 ± 10	59	+ <sup>b</sup>
CAI (B)	0	122 ± 6	100	-
	0.1	126 ± 7	103	-
	0.2	143 ± 9	117	-
Calcium glucarate (B)	0	117 ± 5	100	-
	25	77 ± 3	66	+ <sup>b</sup>
	50	56 ± 4	48	+ <sup>b</sup>
Carbenoxolone (B)	0	182 ± 8	100	-
	0.6	225 ± 21	124	c
	1.2	235 ± 17	129	c
Dihydroepiandrosterone analog 8354 (B)	0	182 ± 8	100	-
	0.1	205 ± 13	113	-
	0.2	133 ± 12	73	+ <sup>b</sup>
Diallyl disulfide (B)	0	237 ± 8	100	-
	0.1	183 ± 11	77	+ <sup>b</sup>
	0.2	230 ± 18	97	-
Diclofenac (B)	0	179 ± 8	100	-
	0.06	131 ± 10	73	+ <sup>b</sup>
	0.13	82 ± 11	46	+ <sup>b</sup>
Difluromethylornithine (B)	0	124 ± 9	100	-
	1	94 ± 13	76	-
	2	100 ± 13	81	-
Folic acid (B)	0	237 ± 8	100	-
	2.5	181 ± 10	76	+ <sup>b</sup>
	5	227 ± 11	96	-
4-Hydroxyphenylretinamide (B)	0	117 ± 5	100	-
	0.39	92 ± 10	79	+ <sup>b</sup>
	0.78	94 ± 6	80	+ <sup>b</sup>
Ibuprofen (B)	0	133 ± 4	100	-
	0.2	115 ± 10	86	-
	0.4	108 ± 6	81	+ <sup>b</sup>
Ketoprofen (B)	0	175 ± 7	100	-
	0.1	99 ± 8	57	+ <sup>b</sup>
	0.2	78 ± 7	45	+ <sup>b</sup>
Meclofenamate sodium (B)	0	179 ± 8	100	-
	0.02	181 ± 10	101	-
	0.04	154 ± 15	86	-
Nicotinic acid (B)	0	179 ± 8	100	-
	0.4	173 ± 12	97	-
	0.8	171 ± 9	96	-
Oltipraz (B)	0	237 ± 8	100	-
	0.1	224 ± 13	95	-
	0.2	244 ± 13	103	-
Piroxicam (B)	0	124 ± 9	100	-
	0.2	49 ± 5	40	+ <sup>b</sup>
	0.4	37 ± 6	30	+ <sup>b</sup>
Quercetin (B)	0	133 ± 4	100	-
	15	74 ± 5	56	+ <sup>b</sup>
	30	70 ± 5	53	+ <sup>b</sup>
All-trans-retinoic acid (B)	0	139 ± 5	100	-
	0.19	46 ± 4	33	+ <sup>b</sup>
	0.39	46 ± 4	33	+ <sup>b</sup>
9-cis-retinoic acid (B)	0	129 ± 6	100	-
	0.19	90 ± 5	70	+ <sup>b</sup>
	0.39	73 ± 5	57	+ <sup>b</sup>
13-cis-retinoic acid (B)	0	139 ± 5	100	-
	0.19	75 ± 5	54	+ <sup>b</sup>
	0.39	66 ± 6	47	+ <sup>b</sup>

**Table II. Cont.**

Agent (protocol)	Dose (g/kg diet)	Aberrant crypts/colon		
		Mean ± SEM	Percent of control	Result
Retinol (B)	0	139 ± 5	100	-
	0.19	108 ± 8	78	+ <sup>b</sup>
	0.39	82 ± 6	59	+ <sup>b</sup>
Ro16-9100 (B)	0	129 ± 6	100	-
	0.19	96 ± 4	74	+ <sup>b</sup>
	0.39	117 ± 6	91	-
Rutin (B)	0	133 ± 4	100	-
	15	81 ± 6	61	+ <sup>b</sup>
	30	41 ± 3	31	+ <sup>b</sup>
S-allylcysteine (B)	0	129 ± 6	100	-
	0.125	115 ± 5	89	-
	0.25	128 ± 5	99	-
SC58635 (B)	0	178 ± 7	100	-
	0.56	151 ± 7	85	-
	1.13	163 ± 8	92	-
Sulindac sulfide (B)	0	193 ± 6	100	-
	1.5/0.5	48 ± 5	25	+ <sup>b</sup>
	3/1	28 ± 7	15	+ <sup>b</sup>
Sulindac sulfone (B)	0	193 ± 6	100	-
	1	112 ± 8	58	+ <sup>b</sup>
	2	121 ± 8	63	+ <sup>b</sup>
Sulindac sulfoxide (B)	0	193 ± 6	100	-
	0.16	85 ± 9	44	+ <sup>b</sup>
	0.32	117 ± 6	61	+ <sup>b</sup>
Theophylline (B)	0	179 ± 8	100	-
	2 mg	222 ± 14	124	c
	4 mg	176 ± 11	98	-

<sup>a</sup>Averages of scorers 1 and 2.<sup>b</sup>+, significant inhibition compared with control group,  $P < 0.05$ .<sup>c</sup>Significantly higher than control group,  $P < 0.05$ .

time of exposure to AOM. The most inhibitory compounds were *p*-chlorophenylacetate, chlorpheniramine maleate, carboxyl amidoimidazole (CAI), D609, diclofenac, ETYA, etoperidone, farnesol, ferulic acid, lycopene, meclizine, *L*-methionine, phenylhexylisothiocyanate (PHITC), sodium phenylbutyrate, piroxicam, 9-cis-retinoic acid, S-allylcysteine, taurine, tolmetin, tetracycline, triprolidine, verapamil and WR151327. These data when combined with those from our previous study continue to suggest that NSAID compounds (e.g. diclofenac and piroxicam), certain plant phenolics or flavonoids (ferulic acid), isothiocyanates (PHITC) and organosulfur compounds (S-allylcysteine) may in some way modulate AOM metabolism or modify early cellular events in the process of aberrant crypt formation. One class of chemicals, the antihistamines, represented in this study by chlorpheniramine maleate, meclizine and triprolidine (but not promethazine), were very effective suppressors of ACF during initiation. Some compounds tested produced mixed dose-effect results, inhibiting at one but not both doses tested. This pattern was found for tea polyphenolics and the farnesylation inhibitors farnesol and geranyl geraniol, among others. Several compounds promoted the development of ACF (increased ACF number or multiplicity) either at one or both doses tested. These included 2-*n*-butylthiophene, carbenoxalone, green and black tea extracts, meclofenamate, *p*-methoxyphenol, nerolidol, 1,2-oxothiazolidine 4-carboxylate and quinacrine HCl.



**Fig. 3.** Effect of selected retinoids on ACF progression. Compounds were given in the diet post-initiation following AOM. Values are means  $\pm$  SD. \*Significantly different from the control at  $P < 0.05$ .

Table II lists the agents that we evaluated in the post-initiation assay. We devised this protocol to address the need for a variation of the assay that would identify possible chemopreventive compounds acting to regress or retard the outgrowth of ACF into microadenomas, precursors to colon cancer. The data suggest that there are two chemical classes of agents that were significantly effective in this respect, the NSAIDs and certain retinoids. From the NSAID class, aspirin, diclofenac, ketoprofen, piroxicam and sulindac and its metabolites sulindac sulfide and sulindac sulfone were strongly inhibitory toward ACF outgrowth and progression into larger lesions. From the retinoid class, 4-hydroxyphenretinamide, all-trans retinoic acid, 9-cis-retinoic acid, 13-cis-retinoic acid and retinol significantly inhibited ACF progression. This effect is also evident in an arrest of outgrowth of ACF into multiple crypt clusters (crypt multiplicity). This is shown for the sulindac compounds in Figure 2 and for representative members of the retinoid class in Figure 3.

## Discussion

In this study we have used two variations of an experimental protocol designed to screen potential chemopreventive agents for efficacy in reducing the incidence of ACF, carcinogen-

induced precursors of colon cancer. ACF are an appropriate target for chemopreventive drug development as increasing evidence suggests them to be in the pathway leading to colon cancer (8–11). Studies characterizing ACF at the molecular and cellular levels implicate ACF as intermediate lesions leading to colon cancer. ACF in rats and humans exhibit defects in DNA content, morphology and proliferation kinetics and mutations have been detected in key genes in the process of colon cancer development (10,12–18). Recent studies also hint that ACF are precursors of colon cancer in humans and this further stimulates the need to identify drugs that may prevent their progression (11,19–21).

In this report we have evaluated a number of compounds for the ability to prevent ACF in the well-studied AOM colon cancer model in the rat. The agents chosen for the study were prioritized by reports of previous activity in the research literature, by a beneficial finding in *in vitro* chemopreventive protocols conducted by the National Cancer Institute or by communication with the National Cancer Institute. The data suggest that during the initiation phase of AOM carcinogenesis two classes of agents are active inhibitors, the antihistamines (chlorpheniramine, meclizine and triprolidine) and a group of plant phytochemicals (ferulic acid, lycopene and S-allylcysteine). We also noted some chemopreventive activity for the differentiation agents *p*-chlorophenylacetate and phenylbutyrate. Chlorpheniramine, meclizine and triprolidine are members of a family of drugs that function as histamine H<sub>1</sub> receptor antagonists and are widely used in the control of allergies (22,23). The results found in our study are not readily explained, although antiproliferative effects have been reported for chlorpheniramine on breast cancer cells *in vitro* and this antihistamine and triprolidine are known to modulate certain cytochromes P450 in rats (24–26). Ferulic acid has been shown to prevent oral and skin cancer in rodents, while lycopene has been implicated as a possible anticarcinogen in human diets and may protect against prostate cancer (26–29). Few chemopreventive experiments with lycopene have been done in animals. PHITC, a synthetic isothiocyanate, and S-allylcysteine from alliums probably exert their effects through modulation of carcinogen metabolism (30,31). PHITC modulates CYP2E1 but has been shown to promote the induction of esophageal carcinogenesis despite earlier reports of chemopreventive activity, whereas S-allylcysteine, an inhibitor of CYP2E1, prevents dimethylhydrazine-induced colon cancer in mice (32–34). The unsubstituted and substituted phenyl fatty acids *p*-chlorophenylacetate and phenylbutyrate both showed chemopreventive effects in the initiation phase assay. Both compounds are powerful differentiation agents and are used in cancer chemotherapy (35). Phenylbutyrate is known to be a potent inducer of apoptosis and agents that strongly induce apoptosis (the NSAIDs for example) are good chemopreventives in the rat colon cancer model (7,36–38).

This study also conducted an in-depth evaluation of agents selected for a high probability of acting as preventives in the post-initiation phase against established ACF. The protocol described may better identify agents for further preclinical evaluation that retard the growth or regress ACF *in vivo*. Such agents would be likely to prevent recurrence of colonic adenoma in humans. From the NSAID class, aspirin, diclofenac, ibuprofen (high dose), ketoprofen, piroxicam and sulindac and its metabolites strongly suppressed ACF incidence and crypt multiplicity. A decrement in crypt multiplicity suggests that the compound may be acting in a cytostatic or

bio-regressive way. Many of the agents we tested in this study have been shown to prevent colon cancer in the long term. Additionally, for the NSAID class in particular, habitual consumption results in a reduced risk for colon cancer (39,40). The study also identified several retinoids as effective suppressors of established ACF, notably 4-hydroxyphenretinamide, all-trans-retinoic acid, 9-cis-retinoic acid, 13-cis-retinoic acid and retinol. Many of these compounds have already entered clinical trials for prevention of tumors other than in the colon (41-47). The results suggest that consideration should be given to the potential use of retinoids for suppression of preneoplasia in the colon. The rodent ACF assay is a powerful screening tool for the preclinical identification of chemopreventive agents with high sensitivity and specificity (48). The findings of this study provide an impetus for further in-depth evaluation of the NSAIDs and retinoids and other compounds in the prevention of colon cancer.

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